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Maintenance of High-Avidity Rubella-Specific IgG Antibody and Titres in Recent HIV Seroconvertors and in Patients Progressing to the AIDS-Related Complex and AIDS

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The avidity (functional affinity) and titre of rubella-specific IgG antibodies were examined in sequential and cross-sectional sera from 38 adult HIV-infected patients, whose HIV status ranged from pre- and recent HIV seroconversion to the AIDS-related complex (ARC) and AIDS, in order to determine whether a preexisting mature antibody response to rubella is maintained or if there is a need for rubella (re)vaccination. Thirty-five patients were already rubella-seropositive and one became rubella-seropositive during the time in which sera were collected. Although the avidity of rubella-specific IgG was higher in HIV-positive patients than in their age- and sex-matched HIV-negative counterparts, the difference was not significant. The titres of this antibody, however, were significantly higher in the HIV-positive patients. No significant decrease in antibody avidity or titre were seen in sequential sera from individual HIV-positive patients except when the titres in pre-HIV-seroconversion sera were compared with the titres in sera from patients with AIDS, where a significant decrease was observed. This would suggest that preexisting humoral immunity to rubella in HIV-infected patients is not compromised with HIV disease progression and there should be no need to revaccinate. ***J. Med. Virol 58:273–279, 1999.***

KEY WORDS: protective immunity; functional affinity

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) leads to a state of increasing immune deficiency in which viruses, which normally cause minimal morbidity, can be life-threatening [Kaplan et al., 1992]. When preexisting antibody does not exist, opinion appears to be that HIV-infected children and adults should be vaccinated against common infectious diseases as soon as possible if these infections could cause serious morbidity or mortality. However, the antibody response to vaccines such as measles [Brena et al., 1993; Brunell et al., 1995], rubella [Brena et al., 1993], influenza [Chadwick et al., 1994], *Streptococcus pneumoniae* [Peters et al., 1994], and tetanus [Barbi et al., 1992] in HIV-positive children and adults [Kroon et al., 1994] is suboptimal. In contrast to the picture after vaccination, others have shown that the levels of antibodies to common viruses, including rubella virus, were within the normal range in longitudinally studied HIV-positive adults when infection or vaccination had occurred before HIV infection [Flo et al., 1993; Brunell et al., 1995].

Unnecessary vaccination should perhaps be avoided, however, in HIV-infected patients since even killed or inactivated vaccines have been reported to increase replication of the human immunodeficiency virus itself [Stanley et al., 1996]. Stimulation of peripheral blood mononuclear cells with CD4-dependent antigens has also been shown to cause apoptosis of CD4+ T cells [Clerici et al., 1996]. However, these results are in conflict with those of others which failed to show these adverse effects [Glesby et al., 1998]. These conflicting results may be due to several factors, including the variable times after vaccination at which viral load was measured, the small size of some of the populations studied and the use/nonuse of antiviral therapy.

Acute, primary rubella infection in pregnancy still occurs and has resulted in 320 terminations of pregnancy and the birth of 146 congenitally infected infants in the UK alone during the period 1986–1996 [Tookey and Peckham, 1999]. Any pregnant HIV-positive woman not already immune to rubella risks rubella infection during pregnancy. Not all females have had

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TABLE I. The Number of Rubella Seropositive Sera Available per HIV-Positive Patient

No. sera available/patient	No. patients	Total no. sera	Time span[a]
			(Range; mean; standard deviation)
1	7	7	18 m, 6.5 years after seroconversion[b]
2	15	30	8 d–45 m; 20.88; 15.3
3	10	30	8 d–51 m; 21.33; 15.9
4	1	4	1 m, 6 m, 2 m
6	1	6	4.5 m, 2.5 m, 2.5 m, 3 m, 2 m
12	1	12	14 d–22.5 m (at 1.5–3 m intervals)
18	1	18	7 d–21.5 m (at 7 d–3.75 m intervals)
Total	*36*	*107*	

[a]Time intervals (not time after seroconversion/illness) between sera in cases where sequential sera were available are given (d = days; m = months).
[b]When only single sera were available (7 patients) the time between seroconversion/illness and the date of the serum was known in 2 cases only.

natural rubella or have received rubella vaccine and, among those who have been vaccinated, a proportion will not have been vaccinated successfully. A rubella IgG antibody level of >15 IU/ml is accepted as protective and once achieved is believed to be maintained for many years [O'Shea et al., 1985]. However, we are aware of only one study on the long-term maintenance of rubella antibody titres in HIV-infected patients, over a period of up to 56 months (mean: 20 months) [Flo et al., 1993]. We are unaware of any studies on the maintenance of high-avidity rubella-specific antibodies in these patients.

The avidity (functional affinity) of an antibody is a measure of the overall strength of interaction between antibody and antigen [Roitt et al., 1998]. Following a primary infection or vaccination, the avidity of specific IgG generally increases and the presence of high-avidity antibodies reflects a mature memory response [Berek and Ziegner, 1993; Thomas et al., 1992; Thomas et al., 1995]. Exceptions to this rule have been found in HIV [Thomas et al., 1996] infection and hepatitis B virus (HBV) infection [Thomas, 1997] where a marked delay or inability to produce high-avidity antibodies to some of the antigens present on these viruses was observed. This inability to develop a high-avidity response in these infections may play a significant role in the pathology of the diseases.

Using an enzyme-linked immunosorbent assay (ELISA) for the assessment of relative antibody avidity routinely used in this laboratory for the serological confirmation of recent primary rubella infection [Thomas and Morgan-Capner, 1988], the avidity of rubella-specific IgG antibodies was compared in symptomatic and asymptomatic HIV-infected adults with the avidity of rubella-specific IgG in age- (±9 months) and sex-matched patients whose sera were found to be HIV-antibody-negative (HIV-negative control group). The method used was one that is more likely to detect low avidity-specific antibody if it is present than others [Thomas and Morgan-Capner, 1991] and could, therefore, be relied upon to detect any differences in avidity should they exist. Using the dilution curves produced in the avidity ELISA the titre of the rubella-specific IgG was also noted.

Ethical Research Committee approval was obtained for this study.

MATERIALS AND METHODS

Sera

Sera from 38 adult patients (36 males, 2 females; aged 24–63 years; mean 32.2 years; standard deviation 9.4 years) infected with HIV, were investigated to determine the relative avidity and titre of their rubella-specific IgG. Where more than one serum was available from a patient, the last serum in each patient series was tested first for rubella-specific antibodies by single radial haemolysis (SRH) [Champsaur et al., 1980] and 36 patients were found to be rubella-seropositive (≥15 IU/ml). Sera from two patients were rubella-seronegative and the patients removed from the study. Then 107 sera from the remaining 36 patients were assessed for rubella-specific IgG status. One of these 36 patients apparently contracted a rubella infection (not documented in case notes) during the period of collection of his sera. The remaining 35 patients were rubella-seropositive from the time of their first available sera. Between 1 and 18 sera taken over a period of time (0–6.5 years) were available from each HIV-positive patient (see Table I).

Sequential sera from the age- and sex-matched HIV-negative and control group were not available and single sera only from individual HIV-negative patients were tested. For statistical analysis of differences between the rubella serology of the two categories, the first serum (or only serum, in cases where only one serum per patient was available) from each HIV-positive case was compared with its matched HIV-negative serum. Statistical analyses were also carried out to determine the significance of changes in antibody avidity or titre in sequential sera taken over time from the HIV-positive cases.

Sera were identified as HIV-positive if they were positive with (1) Behring Anti-HIV-1/-HIV-2 (Behring Hoechst UK Ltd., Hounslow, UK), and (2) Abbott Re-

combinant HIV-1/HIV-2 third generation (Abbott, Maidenhead, UK), then with (3) Serodia HIV passive particle agglutination test (Fujirebio Inc., Mast Group, Bottle, UK), and (4) Liatek line immunoassay (Organon Teknika, Cambridge, UK). They were classified into four groups according to the clinical status of the patients at the time of the blood donation. Group 0 sera were from patients before the appearance of HIV-specific antibodies (but under investigation for HIV status due to contact or HIV-like illness) (3 sera), group 1 from asymptomatic HIV-positive patients (86 sera), group 2 from patients with AIDS-related complex (ARC) (9 sera), and group 3 from patients with AIDS (9 sera).

Clear HIV seroconversion data were available for four patients with both HIV-negative and HIV-positive sera available for three of them. HIV seroconversion times were estimated in a further nine patients from clinical information strongly suggestive of a seroconversion illness. Among single-sera patients one had ARC and one had AIDS at the time the sera were taken. Among patients with sequential sera three had ARC at the initial blood collection. One of these subsequently developed AIDS during the course of the study. Three other sequential-sera patients had AIDS at the onset of the study, another developed ARC and one developed AIDS.

Antibody Avidity Assessment

The rubella-specific IgG-positive sera from every single-serum HIV-positive case, and the first sera from the sequential-serum HIV-positive cases were tested, in parallel, with rubella-specific IgG-positive sera from age- and sex-matched HIV-negative patients. All assays included routinely used high- and low-avidity antibody control sera from healthy individuals (no known risks for HIV infection and not tested for HIV).

The method for rubella IgG avidity assessment has been published elsewhere [Thomas and Morgan-Capner, 1988]. Briefly, an adapted enzyme-linked immunoassay (ELISA) was carried out with wells of Polysorp microtitre plates (Nunc, Gibco BRL, Life Technology Ltd., Paisley, UK) coated with a predetermined optimum concentration of rubella antigen (produced for the in-house rubella M-antibody capture radioimmunoassay [MACRIA] by the Public Health Laboratory Service, 61 Colindale Avenue, London NW9 5HT). Sera were added to the rubella-coated wells and diluted, in parallel, in PBST containing 5% normal goat serum (Sera-Lab Ltd., Crawley Down, W. Sussex, UK) (5%NGS/PBST) only, and in 20 mM diethylamine (DEA) (BDH, Poole, Dorset, UK) in 5%NGS/PBST (DEA/PBST) as described previously. Preliminary tests were carried out to ensure that <5% of the antigen was removed from the wells of the plates by the DEA.

Graphs of the optical density (OD) against the reciprocal of the log of the serum dilution were drawn for each pair of serum dilutions (one in 5%NGS/PBST and one in DEA/PBST) and the distance apart at the midpoint on the linear section of the graph measured to give the DEA shift value (DSV). The mean (x) DSV and the standard deviation (SD) for the internal control high-avidity sera were calculated. The results of any assay in which the mean for the high-avidity controls was not within 2 SD of the values of the high-avidity controls used in the routine laboratory or in which the DSV of the acute control was not $>x + 5$ SD were discarded and the assay repeated. Criteria used in the routine laboratory were applied and a serum was considered positive for low-avidity rubella-specific IgG if its DSV was $>x + 5$ SD, equivocal for low-avidity antibody if its DSV was $\geq x + 3$ SD but $\leq x + 5$ SD, and negative for low avidity antibody (i.e., contained mainly high-avidity antibody) if its DSV was $<x + 3$ SD.

The titre of each HIV-positive- and HIV-negative-matched serum was noted in the ELISA as the reciprocal of the log at a predetermined cutoff OD of 0.100 using the serum dilution series prepared in 5%NGS/PBST (no DEA).

Statistical Analysis

The Arcus Quickstat package was used for statistical analysis of the results.

RESULTS

Overall Rubella Seropositivity

Of the 38 HIV-positive patients, 35 were already rubella-seropositive at the time of their first serum donation for this study, giving a rubella seroprevalence of 92%. One other patient developed rubella-specific IgG between his second and third donation so increasing the prevalence to 95%.

Rubella-Specific IgG Antibody Avidity

None of the sera, either from the HIV-positive cases or from the age- and sex-matched HIV-negative sera, contained low-avidity rubella-specific IgG antibody. Eight HIV-positive and five HIV-negative sera gave equivocal results. The rubella IgG-positive sera from the HIV-positive patient who apparently contracted a rubella infection also contained high-avidity rubella-specific IgG. There was a 6-year interval between the last rubella-negative serum and the first rubella-positive serum from this patient.

There were no significant differences between different clinical status groups and rubella-specific IgG antibody avidity (DSV results) when the Mann-Whitney U test was applied:

Group 0 (pre-HIV seroconversion) compared with group 1 (asymptomatic HIV-positive), $P = .897$; compared with group 2 (ARC), $P = .973$; compared with group 3 (AIDS), $P = .482$.
Group 1 compared with group 2, $P = .429$; compared with group 3, $P = .208$.
Group 2 compared with group 3, $P = .287$.

When the avidity of rubella-specific IgG antibodies in sera from the HIV-positive patients (single sera and first sera from sequential serum series) was compared with the avidity of rubella-specific IgG antibodies in

TABLE II. Breakdown of the Clinical Status of HIV-Positive Patients, From Whom Sequential Sera Were Available, at the Time of Donation of Their "First" and "Last" Sera

	Clinical status[a]				
Sera	0	1	2	3	Total
"First"	3 (10%)	21 (72%)	3 (10%)	2 (7%)	29
"Last"	0	21 (72%)	4 (14%)	4 (14%)	29

[a]Clinical status groups: 0, pre-HIV-seroconversion patients; 1, asymptomatic HIV-positive patients; 2, AIDS-related complex (ARC) patients; 3, AIDS patients.

sera from age- and sex-matched HIV-negative patients, no significant difference was found (mean DSV of HIV-positive sera 0.463 (SD 0.175); mean DSV of matched HIV-negative sera 0.525 (SD 0.151); $P = .0551$; Paired t test) although the mean DSV in the HIV-positive sera was lower (suggesting higher avidity antibodies) than in the HIV-negative sera.

Rubella-Specific IgG Antibody Avidity Changes in Sequential Sera From HIV-Positive Cases

Sequential sera were available from 29 HIV-positive patients. The profile of the clinical status of the patients at the time of donation of their "first" and "last" sera is given in Table II. When the avidity of the rubella-specific IgG antibody in the first and last sera from each patient series was compared, although there were some differences (see Fig. 1), they were not significant (mean DSV of first sera 0.475 (SD 0.192); mean DSV of last sera 0.454 (SD 0.150); two-sided $P = .678$, Wilcoxon's signed ranks test). The time between the first and last sera was not the same for every patient.

Figure 2 shows the rubella-specific IgG avidity (DSV) results in relation to time after HIV seroconversion. Sera were available from four patients with a clear HIV seroconversion history and from nine others who had a dated seroconversionlike illness. No correlation between the avidity of the rubella-specific IgG and time after HIV seroconversion was noted (Linear regression: standard error of slope = 0.0179; 95% CI for population value of slope = −0.039 to 0.034; Correlation coefficient (r) = −.025 ($r^2 = .0006$); Two-sided $P = .877$).

Rubella-Specific IgG Antibody Titres

While there was a general decrease in rubella-specific IgG antibody titre as the clinical status of the HIV patients progressed towards AIDS, the only significant difference was observed when sera from clinical status groups 0 and 3 were compared ($P = .009$, Mann-Whitney U test). The mean (and standard deviation) titres for the different clinical status groups was: 4.233 (0.404) for group 0; 3.908 (0.498) for group 1; 3.77 (0.76) for group 2; 3.733 (0.117) for group 3.

When the titres of rubella-specific IgG antibodies in sera from the HIV-positive patients (single sera and first sera from sequential serum series) were compared with the titres of rubella-specific IgG antibodies in sera from age- and sex-matched HIV-negative patients, a significant difference was found (mean titre HIV-positive sera 4.027 [SD 0.444]; mean titre matched HIV-negative sera 3.839 [SD 0.376]; $P = .0288$, paired t test) with the mean titre in the HIV-positive sera higher than in the age- and sex-matched HIV-negative sera.

Rubella-Specific IgG Antibody Titre Changes in Sequential Sera From HIV-Positive Cases

Although there were some differences in rubella-specific IgG antibody titres between the first and last sera from these patients (see Fig. 3), with the mean titre in the first sera being slightly higher than in the last sera, the differences were not significant (mean titre first sera 4.010 (SD 0.422); mean titre last sera 3.910 (SD 0.435); two-sided $P = .123$, Wilcoxon's signed ranks test).

Figure 4 shows the rubella specific IgG titre results in relation to time after HIV seroconversion. No correlation between the titre of the rubella-specific IgG and the time after seroconversion was noted (linear regression: standard error of slope = 0.0496; 95% CI for population of slope = −0.046 to 0.155; $r = .175$ ($r^2 = .0306$) two-sided $P = .2804$).

DISCUSSION

The maintenance of the humoral response to rubella, a virus likely to have been encountered by the majority of adult patients before infection with the human immunodeficiency virus, either by natural infection or by vaccination, was studied here in a group of symptomatic and asymptomatic HIV-positive patients. Rubella is not a latent virus and the humoral immunity produced as a result of earlier exposure to the virus in HIV-positive patients does not appear to be compromised. We are unaware of any reports of complications due to rubella reinfection in such cases. Viruses such as CMV, on the other hand, can cause serious morbidity in these patients [Chan et al., 1995].

Our results show that the majority of patients with HIV infection were already immune to the rubella virus; 35 of 38 patients had SRH values >15 IU/ml in their first sera available for this study. The results also show that, while the avidity of these antibodies is not significantly different from the avidity of the rubella-specific IgG antibodies detected in age- and sex-matched HIV-negative patients ($P = .0551$, paired t test), their avidity is, if anything, slightly higher (DSV lower, indicating higher avidity antibodies) than in the HIV-negative sera. These results agree with those of Brunell et al. [1995] who showed that the avidity of measles-specific antibodies acquired prior to HIV infection in adults was not significantly different from that seen in the normal population while HIV-positive infants immunised with measles vaccine produced low titres of lower than normal avidity measles-specific antibody. Our results also show that high avidity rubella-specific IgG antibodies continue to persist in HIV-positive cases even when the disease progresses to ARC and AIDS and no significant differences were found in antibody avidity when pre-HIV-seroconversion sera

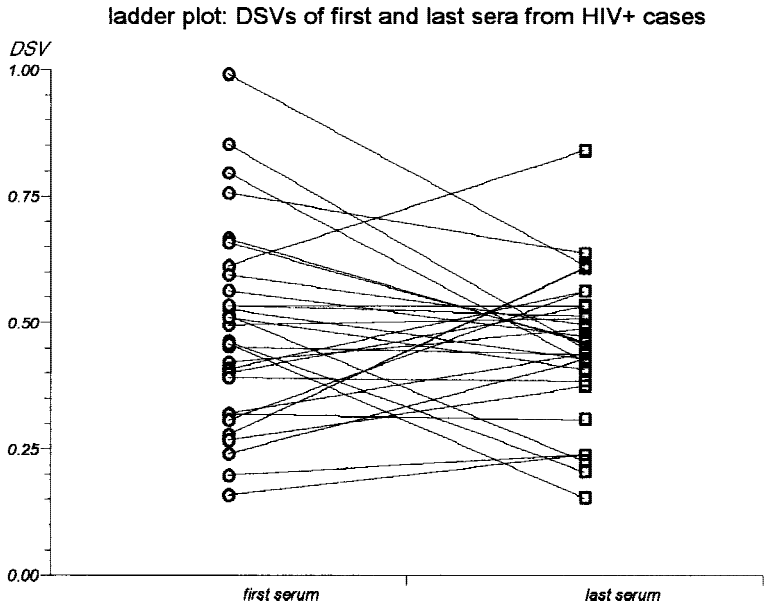

Fig. 1. Ladder plot of rubella-specific IgG avidity (DSV) in "first" and "last" sera from HIV-positive patients from whom sequential sera were available. Mean DSV of "first" sera 0.475 (SD 0.192); mean DSV of "last" sera 0.454 (SD 0.15); two-sided $P = .678$, Wilcoxon's signed ranks test. The time intervals between 'first' and 'last' sera was not the same in every case.

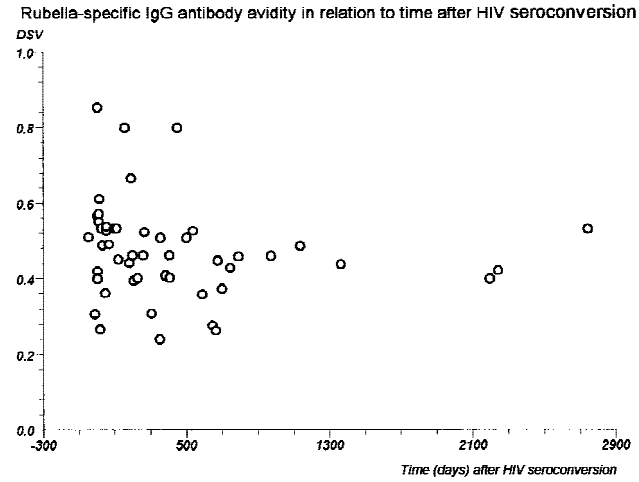

Fig. 2. Rubella-specific IgG antibody avidity (DSV) in relation to time (days) after HIV seroconversion or seroconversion illness. Linear regression: standard error of slope = 0.0179; 95% CI for population of slope = −0.039 to 0.0335; correlation coefficient (r) = −.025 (r^2 = .000637); Two-sided $P = .877$).

were compared with asymptomatic HIV-positive sera ($P = .0.897$), with sera from cases of ARC ($P = .973$) or from cases of AIDS ($P = .482$). It was also demonstrated that no significant changes occur in rubella-antibody avidity in first and last sera from patients with sequential sera ($P = .678$). Furthermore, as this technique has been shown to be more sensitive at detecting low avidity antibodies [Thomas and Morgan-Capner, 1991] than the technique used by Brunell and colleagues (1995), these results can more conclusively confirm the maintenance of high-avidity-specific antibody in these cases.

Only one of the patients in this study appeared to have suffered a primary rubella infection after he became HIV-seropositive. The first two sera from this case were rubella-seronegative by SRH, latex and an in-house rubella IgG ELISA. The next serum, taken 6 years later, and 3 months before the onset of ARC, contained rubella-specific IgG of high avidity. Thus it would appear that, in this case at least, an HIV-infected adult was capable of mounting a mature, high-avidity response to this viral infection. The lack of sera over a period of 6 years between the rubella-seronegative specimens and the seropositive ones unfortunately precludes us from determining when the infection occurred (and hence his clinical and immunological status at the time of infection) and whether the maturation rate was the same as that seen in nonimmunocompromised cases [Thomas et al., 1992] or vaccinees [Thomas et al., 1995].

Our results show that the titre of rubella-specific IgG antibodies is actually higher in the HIV-positive patients than in their age- and sex-matched HIV-negative counterparts ($P = .0288$) and that there is little longitudinal change in the titre ($P = .123$) when the first and last sera from sequential sera are compared. However, there does appear to be a significant fall in titre when pre-HIV-seroconversion sera are compared with sera from cases of AIDS ($P = .009$); titres in pre-HIV-seroconversion sera were not significantly different from those in sera from asymptomatic cases of HIV ($P = .348$) nor from those in sera from patients with ARC ($P = .346$).

From these results it was shown that HIV-positive patients appear to be able to maintain a mature T-cell-dependent immunological memory response, at least to a nonlatent virus (rubella), despite progression toward ARC and AIDS, if this response was established before infection with HIV. We cannot draw conclusions from the one case of apparent rubella infection observed during this study.

It would be interesting to carry out further studies on the avidity of specific antibodies in infections which commonly recrudesce in ARC and AIDS such as HSV,

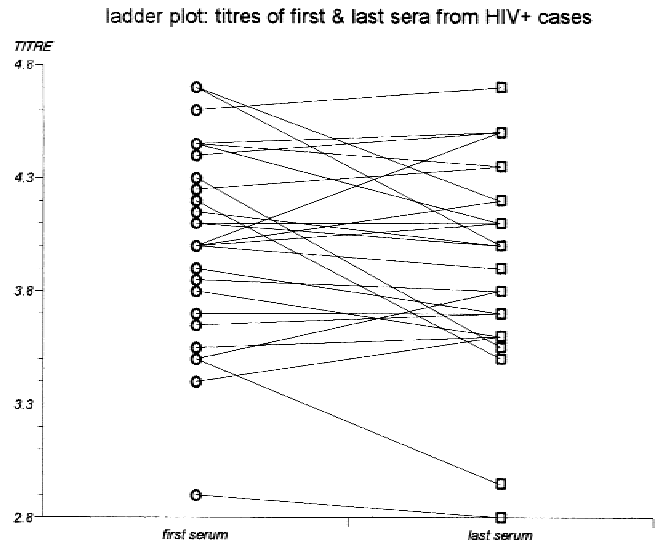

Fig. 3. Ladder plot of rubella-specific IgG titres (reciprocal $\log_{10}$) of "first" and "last" sera from HIV-positive patients from whom sequential sera were available. Mean titre of "first" sera 4.01 (SD 0.422); mean titre of "last" sera 3.910 (SD 0.435); two-sided $P = .123$, Wilcoxon's signed ranks test. The time intervals between 'first' and 'last' sera was not the same in every case.

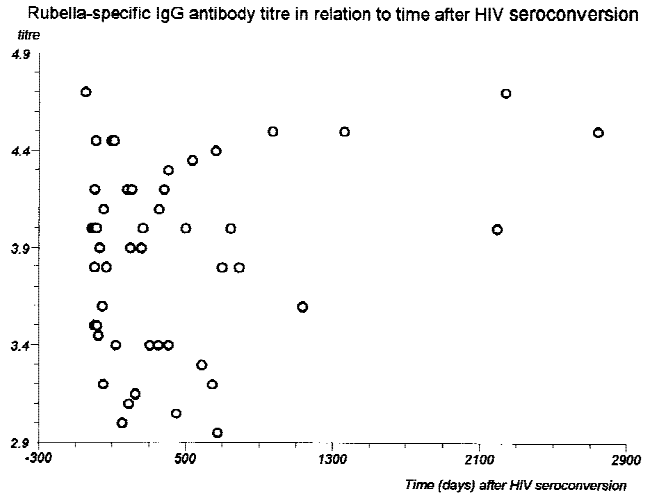

Fig. 4. Rubella-specific IgG antibody titre (reciprocal log) in relation to time (days) after HIV seroconversion or seroconversion illness. Linear regression: standard error of slope = 0.0496; 95% CI for population of slope = −0.0461 to 0.15472; correlation coefficient (r) = −.1749 (r^2 = .030586); two-sided P = .2804).

CMV, VZV, and *Toxoplasma*. Methods for the assessment of specific antibody avidity are already available for CMV [Leydon and Locarnini, 1992], for VZV [Thomas et al., 1990] and for *Toxoplasma gondii* [Holliman et al., 1994].

The findings have implications for the clinical management of HIV-positive patients. HIV appears to replicate more efficiently in activated cells and stimulation by infections or vaccines may cause increased viral load [Stanley et al., 1996] although this has been disputed [reviewed in Glesby, 1998]. Furthermore, antigen-stimulated apoptotic T-cell death has been observed in HIV-positive patients following stimulation by CD4-dependent antigens such as influenza A virus [Clerici et al., 1996] and tetanus toxoid [Farber et al., 1996]. It would seem, therefore, inadvisable to immunise HIV-positive patients against potential infectious agents if there is evidence of an existing effective humoral immunity. On the basis that high avidity specific antibody is more efficient and protective than low avidity antibody [Steward et al., 1991; Berek and Ziegner, 1993; Roitt et al., 1998], our results indicate that rubella reinfection is not likely to be more of a potential risk in HIV-positive patients with pre-existing rubella-specific antibody than in the rest of the community. This should be useful for the clinical management of the HIV-positive patient, especially the HIV-positive pregnant woman.

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